In search of the best performing *Saccharomyces cerevisiae* strain isolated from natural or industrial habitats for the production of bioethanol

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Introduction

Industrial bioethanol production using second generation feedstock like wheat and rice straw, corn stover, sugarcane bagasse etc. is of great commercial interest and necessitates the introduction of yeast strains able to perform under undefined, potentially harsh conditions. Current strains, however, are not selected to resist the wide variety of stressors commonly encountered in such fermentations. In addition, the appropriate tools and knowledge to select such multi-stress tolerant microorganisms and to make a scientifically proven choice of the appropriate candidate strains are lacking. In this study, we performed a phenotypic evaluation of a large yeast culture collection, consisting of 373 *Saccharomyces* strains from diverse origins, for tolerance to different desirable traits for bioethanol production using a high throughput plating robot by recording the growth on solid agar plates. Osmotolerance (glucose, fructose, sorbitol up to 70%), halotolerance (NaCl, KCl, LiCl), thermotolerance (24°C-41°C), ethanol tolerance (5%-15%), tolerance to zinc, copper and cadmium and tolerance to HMF (2-7 g/l) were evaluated.

Experimental set-up and results

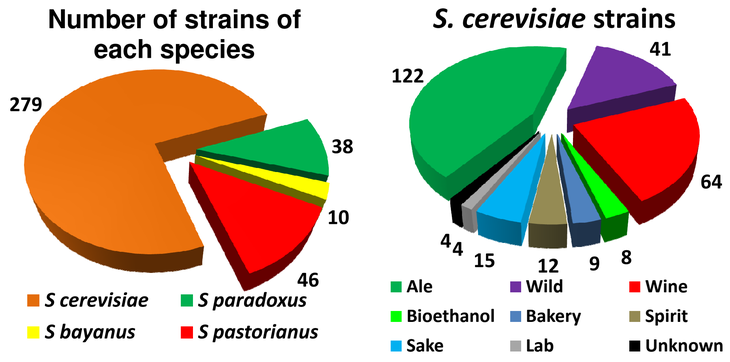


Figure 1: Number of strains of each Saccharomyces species and isolation source of *Saccharomyces cerevisiae* strains in the culture collection

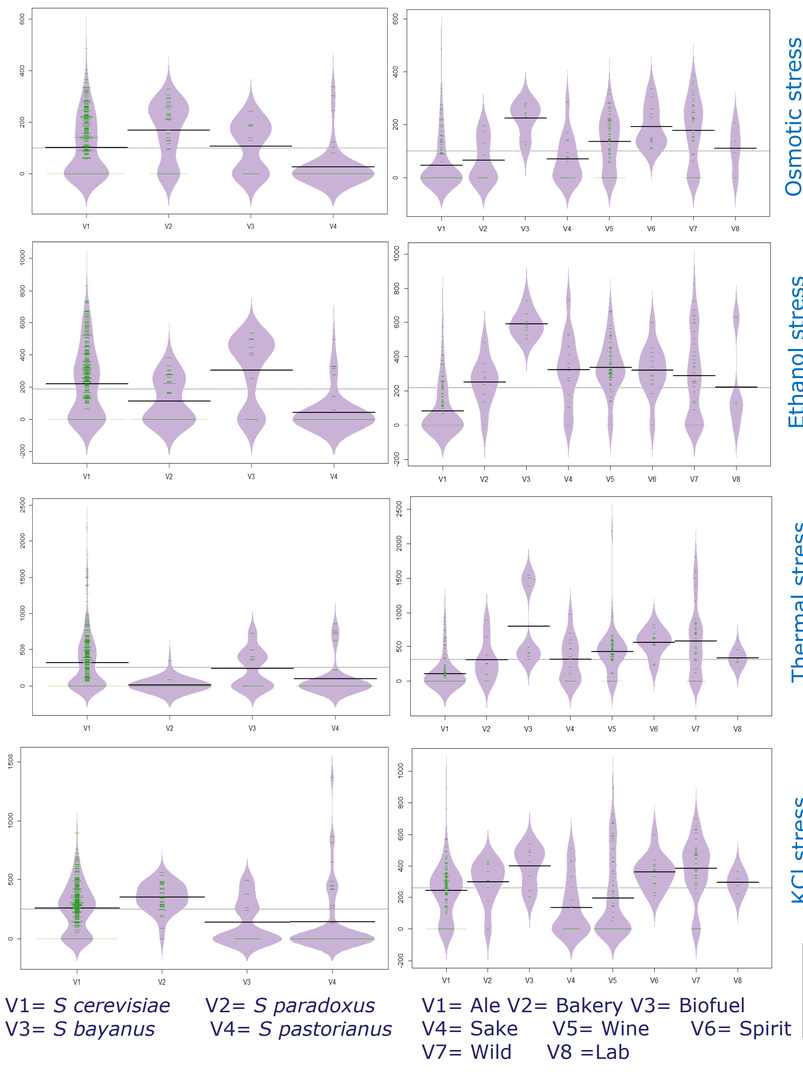


Figure 2: Bean plots show a large phenotypic diversity among different *Saccharomyces* species and among *S. cerevisiae* strains from different origins. Values of the control condition are set to 1000.

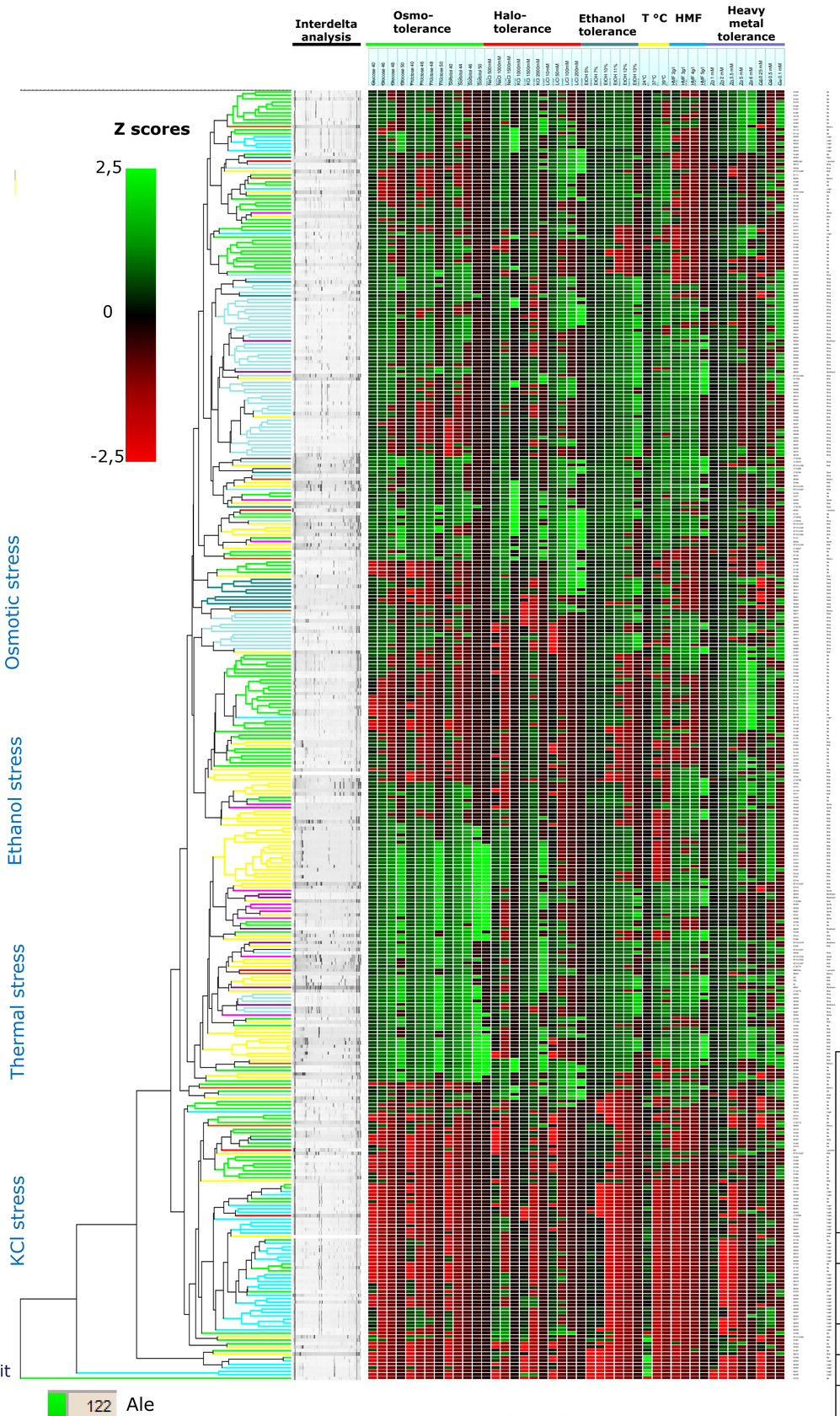
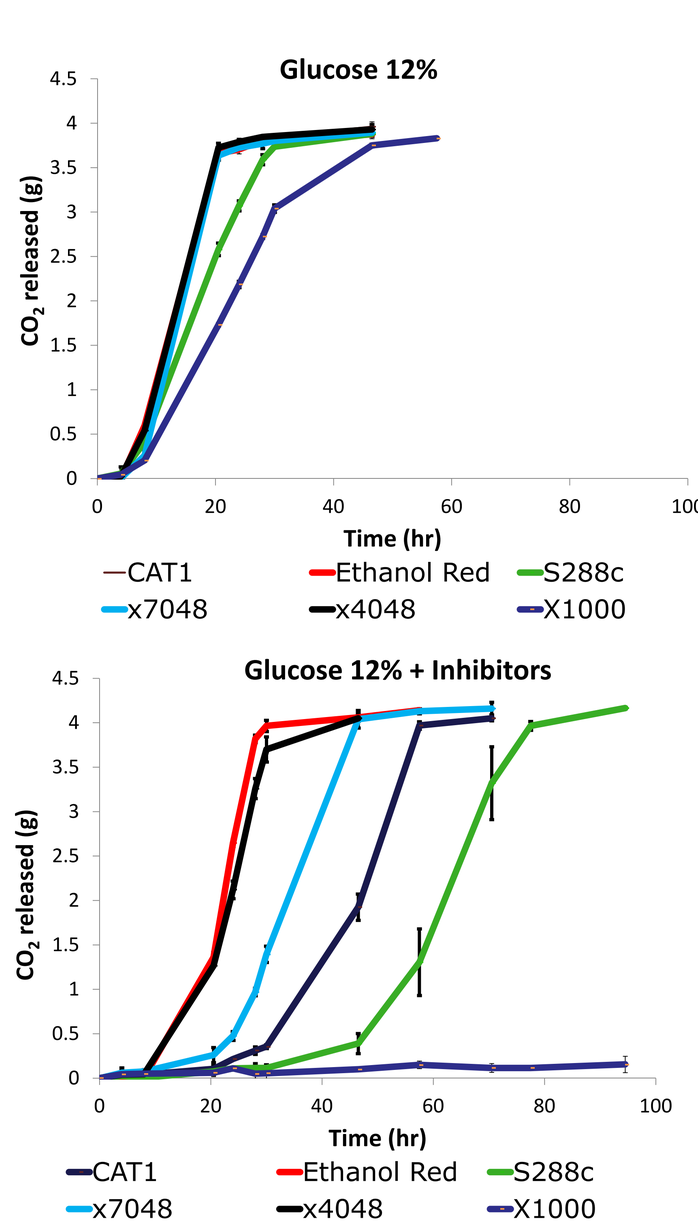


Figure 3: Phenotypic profile of the *Saccharomyces* strains in the screened collection. Phenotypic clusterings were done based on Pearson correlation and UPGMA. Three clusters of ale strains are clearly visible. Lager, wine and wild strains each made one large and one small cluster. One cluster of sake strains is also clearly visible.



	Glucose 12%		Glucose 12% + Inhibitors	
	Ethanol yield/ 100g of sugar	% Theoretical Yield	Ethanol yield/ 100g of sugar	% Theoretical Yield
CAT1	49.33	96%	48.58	95%
Ethanol Red	49.02	96%	51.48	101%
x4048	50.46	99%	50.17	98%
x7048	50.44	99%	48.29	94%
x1000	48,23	94%	2,10	4%
S288c	51.37	100%	49.52	97%

Figure 4: Comparative fermentation efficiency of the multitolerant yeast strains x7048 and X4048 compared to currently used industrial bioethanol strains Ethanol Red ® and CAT1. Two non multitolerant strains S288c and X1000 were also included.

Conclusions

- This study shows the potential of high throughput phenotypic evaluation and subsequent data analysis to characterize a large microbial collection.
- Large phenotypic diversity among strains of different *Saccharomyces* species, and among *S. cerevisiae* strains from diverse origins was observed (Figure 2).
- In Figure 3 phenotypic clusters can be observed for ale (3 clusters), lager, wine and wild strains (1 major and 1 minor cluster for each).
- *S. pastorianus* showed to be the most sensitive to most of the traits compared to the other species.
- Generally, *S. cerevisiae* from ale fermentations were most sensitive to most parameters compared to *S. cerevisiae* strains from other origins
- Among the most multitolerant strains mainly wine, bioethanol and wild *S. cerevisiae* strains from oak were represented
- Fermentation experiment showed that the selected multitolerant strains performed better than industrial strain CAT1, and equal to industrial strain Ethanol Red® in bioethanol fermentations

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